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**Leukocytes, inflammation, and cardiovascular diseases**

Leukocytes have occupied center stage as protagonists in host defenses and inflammatory diseases since the era of the 19th century pioneers including Virchow, Cohnheim, and Metchnikoff.[1] These investigators helped establish the scientific basis of modern medicine through their careful morphologic observation, experimental manipulations, and deductive reasoning. The romance of the white corpuscle achieved popular culture status in the beginning of the 20th century, as reflected by George Bernard Shaw's exhortation to "stimulate the phagocytes" in his 1906 play "The Doctor's Dilemma." [2]

Application of the tools of biochemistry and cell biology enabled fleshing out the functions of phagocytes in the 20th century. Such studies discovered the ability of leukocytes to produce reactive oxygen species (ROS), lipid mediators, and cytokines, as well as granular constituents including plentiful proteinases. This potent armamentarium equips leukocytes to fight invaders, or when turned against the host, to produce or promote disease.

The field of leukocyte biology has flourished during the last several decades. We now possess detailed knowledge of the way in which various leukocyte sub-classes enter, tarry within, and exit the vasculature and tissue compartments (Figure). We have learned what signals beckon these cells to extravasate, and

delineated detailed mechanisms by which they do so. Leukocytes not only enter tissues, but persist within them in response to specific signals, and may in some cases emmigrate, each step subject to regulation by various interesting mechanisms and paths.

The special issue of *Cardiovascular Research* devoted to vascular inflammation edited by Markus Sperandio, Alexander Zarbock, and Klaus Ley includes a series of authoritative and detailed contributions, most of which speak to the functions of leukocytes and other blood cells in host defenses and disease. To place the individual articles into a broader context, the Figure attempts to integrate the roles of two major classes of leukocytes, the granulocyte and the monocyte/macrophage, into a temporal and spatial framework. While grossly oversimplified, the Figure depicts the role of the neutrophil as a mediator of acute inflammation that plays out over hours. In juxtaposition, mononuclear phagocytes, in the cardiovascular system and also other chronically diseased tissues (consider a granuloma in a tuberculous lung), persist in tissue spaces for days to years. Despite the vast differences in the timescales with which these leukocytes do their jobs, their recruitment and migration share many common themes. Yet, the fate of these different leukocyte classes varies considerably (Figure). Although polymorphonuclear leukocytes and monocyte/macrophages generally receive the

lion's share of attention, other leukocyte classes also participate in host defenses and disease including mast cells, basophils, and eosinophils. Although tradition views the platelet as a "thrombocyte", we now recognize platelets as active participants and amplifiers of local inflammatory responses. Platelets often associate with leukocytes, as discussed in the contribution by Rossaint and Zarbock in this compendium.

Beyond the classification of different leukocyte lineages, the last decade has witnessed an explosion in understanding the heterogeneity of leukocytes within a given group.[3 4] Virtually every leukocyte family includes various sub-types that subserve distinct functions and respond to and elaborate different, but often overlapping, patterns of mediators.

While the traditional view of leukocyte participation in inflammation portrays them as phlogistic proagonists, some leukocyte sub-types appear to quell or moderate inflammation. Examples of such leukocytes that can limit inflammation or promote repair include Th2 lymphocytes, regulatory T cells (Treg) M2 macrophages, and Ly6c$^{lo}$ monocytes. While some of the muting of inflammation mediated by certain leukocyte sub-types derives from the production of anti-inflammatory mediators such as IL-10 or transforming growth factor beta, recognition has increased that specific pathways of inflammation resolution,

distinct from anti-inflammatory mediators, usually involving lipids, can help limit inflammatory responses. [5, 6]

**The translational challenge of inflammation in cardiovascular diseases**

Recent advances in leukocyte biology such as those described in the articles herein have unquestionably provided key new insights into pathophysiologic mechanisms. Our community's reports of experimental results in this field often end with a promissory note regarding translation. Yet, a gap yawns between the elegant scientific findings such as those reported in the collection of articles in this issue, and the reduction to practice. Where do we stand in targeting inflammation in cardiovascular disease? (Table) Despite the indubitable involvement of leukocyte recruitment in ischemia-reperfusion injury, scant therapeutic success has emerged from decades of attempts to target leukocyte recruitment in this context. The non-steroidal anti-inflammatory agents, in particular the cyclooxygenase-2 selective members of this drug class, do not improve cardiovascular outcomes, rather to the contrary. The non-selective NSAIDS cause slight degrees of hypertension, which over time may wreak ravages in the cardiovascular system. The COX-2 selective inhibitors may tip the balance of prostanoid production towards excessive pro-aggregatory and vasoconstrictor prostaglandins. Glucocorticosteroids have potent and widespread anti-inflammatory effects, but

their use entails numerous undesired actions that could augment cardiovascular risk including hypertension, insulin insensitivity, and obesity. Aspirin provides protection against a second heart attack, and in some populations, stroke. But the doses of aspirin that exert beneficial cardiovascular actions fall far below the doses required for a true anti-inflammatory effect. By impeding platelet activation, however, even "cardioprotective" doses of aspirin may exert an indirect anti-inflammatory action, a conjecture that requires further investigation.

On the more positive side of the balance sheet, the statin class of hydroxymethylglutaryl co-enzyme A inhibitors resoundingly reduces cardiovascular event rates, but the extent to which they do so by anti-inflammatory functions distinct from lowering of low-density lipoprotein cholesterol remains controversial. A small study showed an impressive reduction in cardiovascular events with treatment with colchicine, an agent that interferes with leukocyte functions.[7] This concept requires rigorous evaluation in a larger double-blind clinical trial. Methotrexate in low doses on a weekly dosing regimen has revolutionized the treatment of rheumatoid arthritis. A large clinical trial currently underway will evaluate its ability to reduce recurrent cardiovascular events in individuals at high risk.[8] Another trial targets p38 MAP kinase, a key participant in the control of inflammation, in secondary prevention of atherosclerotic events.[9]

Anti-cytokine treatment has also proven highly beneficial in some inflammatory diseases such as rheumatoid arthritis. Yet, the anti-tumor necrosis factor antibodies associated with a signal for worsened outcome when studied in large clinical trials for heart failure. A large-scale clinical trial currently underway tests whether a monoclonal antibody that targets interleukin-1-beta might improve outcomes in survivors of myocardial infarction with persistent inflammation (despite contemporary standard of care treatment), as gauged by increased levels of the inflammatory biomarker C-reactive protein.[10]

In sum, while inflammatory pathways such as those discussed in this series of articles remain enticing targets for therapeutic intervention, the promise remains unfulfilled. Given the redundancy of inflammatory pathways, targeting any one mediator may not suffice to limit cardiovascular inflammation in a meaningful way. On the other hand, intervening in pathways that participate in host defense runs the risk of impairing tumor surveillance or the ability of the host to combat infection. Thus, application of the growing body of detailed mechanistic insight we are acquiring regarding pathways of cardiovascular inflammation, the therapeutic application will require finding the "sweet spot," where we selectively target the pathological pathway without impairing host defenses. Expanding knowledge of the fundamental mechanisms of inflammation provides the necessary foundation in

this regard. The clinical translation of the scientific advances reported in this compendium will require considerable rigor and resources, yet remains a goal to which we must aspire.

Table:

## Status of Anti-Infammatory Agents in Atherothrombotic Disease

**Anti-inflammatory strategies less likely to improve cardiovascular outcomes:**
Corticosteroids
Non-steroidal anti-inflammatory drugs
Anti-TNF agents

**Anti-inflammatory strategies that might improve cardiovascular outcomes:**
Colchicine - LoDoCo trial [7], PROBE design, n=532

**Anti-inflammatory strategies under evaluation in cardiovascular outcome trials:**
Methotrexate (low dose weekly) – CIRT [8], RCT, n=7,000 (enrolling)
Anti-Interleukin-1 beta (canakinumab) – CANTOS [10], RCT, n= 10,060 (enrolled)
p38 MAP kinase inhibitor (losmapimod) – LATITUDE/TIMI-60 [9], RCT, n=25500, (enrolling)

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TNF: tumor necrosis factor

PROBE: prospective randomized open blinded end-point

RCT: randomized clinical trial

**Figure legend:**

Panel a shows a simplified "lifecycle" of neutrophil responding to an acute inflammatory or injurious stimulus. The role of the neutrophil typically plays out in a matter of hours. The neutrophil can exercises functions intravascularly, in interstitial spaces, in walled – off collections (abscesses), or within the parenchyma of a tissue. After recruitment and activation, neutrophils release many mediators, in the context of cardiovascular disease notably reactive oxygen species including hypochlorous acid (HOCl) produced by myeloperoxidase (MPO.) The activated granulocyte can elaborate multiple lipid mediators of inflammation. In addition neutrophil granules contain proteolytic enzymes including neutrophil elastase (NE), cathepsin G (CatG), and proteinase 3 (Pr3.) The fates of this short-lived inflammatory cell includes classical apoptosis. The apoptotic cells and debris can be cleared by efferocytosis. In addition, the membrane of neutrophils and fragment, the histones of chromatin can undergo modification by deaminating enzymes that free DNA to spew forth into the extracellular space forming neutrophil extracellular traps (NETs), a process known as "NETosis."

Panel b shows a simplified life history of a mononuclear phagocyte. Monocytes recruited into tissues mature into macrophages. These cells exert phagocytic activity, and also elaborate a myriad of mediators of inflammation. The macrophage can replicate within tissues or die, including by apoptosis. Mononuclear phagocytes may also emigrate into the lymph or blood. These processes generally play out over days or even years.

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